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Progress Report
Natural History of Plexiform Neurofibromas in NF1
DAMD17-98-1-8611

PI: Bruce R. Korf, M.D., Ph.D.

October 31, 2002

Introduction

This report marks completion of the fourth year of this project. The backbone of the study is the recruitment of individuals with NF1 who have plexiform neurofibromas and the use of volumetric MRI to measure the rate of growth of their tumors. Recruitment was significantly delayed by challenges in obtaining IRB approval for the multiple study recruitment sites, although we have developed a stable group of active participating centers. We have demonstrated that volumetric analysis can be accomplished reproducibly, and have a paper in press on this topic. In addition, a study has been done comparing the imaging characteristics of superficial *vs.* deep plexiform neurofibromas. We are now at a point where an increasing number of participants have had multiple MRI scans, permitting the primary aim of the study, *i.e*, measurement of plexiform neurofibroma growth rate, to be initiated. Final data analysis will not be possible until all of the participants have had three MRI's over three years. In order to insure that data analysis continues, it will be necessary to discontinue further enrollment, focusing now on continued accrual of data from enrolled participants and data analysis.

Progress Report for Statement of Work by Task

Task 1. **Complete development of study infrastructure – Months 1-6**

a. **IRB approval at all clinical sites**

Table 1 lists the participating clinical centers, the principal investigator at each site, and the IRB approval status. The IRB column refers to approval by the local IRB; the "Army" column refers to approval by the army IRB. The Mayo Clinic has been added as a new center during this past year.

Center	PI	# Pts	IRB	Army
Children's Hospital Boston - 107	Bruce Korf	22	☑	☑
Children's Hospital Medical Ctr - 173	Robert Hopkin	7	☑	☑
Children's Hospital of Oklahoma - 178	John Mulvihill	12	☑	☑
Children's Memorial Hospital - 177	Joel Charrow	10	☑	☑
Children's National Medical Ctr- 170	Roger Packer	23	☑	☑
Guy's Hospital - 187	Rosalie Ferner	14	☑	☑
Klinikum Nord Ochsenzoll - 160	Victor-Felix Mautner	37	☑	☑
Mass General - 106	Mia MacCollin	3	☑	☑
Mass General - 189	Bruce Korf	4	☑	☑
Mayo Clinic	Dusica Babovic	0	☑	☑
National Cancer Institute - 181	Brigitte Widemann	19	☑	☑
New Children's Hospital - 112	Kathryn North	16	☑	☑
Texas Children's Hospital - 172	Sharon Plon	4	☑	☑
University of British Columbia - 100	Jan Friedman	13	☑	☑
University of Utah - 117	David Viskochil	15	☑	☑
Washington University - 169	David Gutmann	20	☑	☑

Table 1. Status of IRB approval of participating clinical centers.

b. **Complete clinical data entry forms and test electronic transfer of clinical data**

Data entry forms were completed by the end of the first year, and have not changed.

c. **Organize package of materials for pathology review and tissue repository**

This task was completed by the end of the second year and has not changed. A detailed protocol for submission of tissue specimens has been produced and is available for download on our website (www.nfstudies.org).

d. **Set up listserv and website**

The study website has been operational for over two years at www.nfstudies.org. There have been no significant changes in the site over the past year.

e. **Test MRI data transfer**

Each center has submitted test data for the NF1 Study either by optical disk or through File Transfer Protocol (FTP).

f. **Purchase workstation and prepare data entry forms at WorldCare.**

The workstation was purchased in November of 1998. Documentation was provided in last year's progress report.

g. **Prepare project monitoring flow sheet at Brigham and Women's Hospital**

This was addressed last year and no changes have occurred since this time.

h. **Prepare recruitment letters for study subjects**

This was addressed last year and no changes have occurred since this time.

i. **Publicize study to NF community**

The study continues to be publicized in newsletters of the National Neurofibromatosis Foundation and of NF, Inc.

Task 2. ***Recruitment of Study Subjects*** **– Months 6-12**

a. **Centers contact prospective study subjects**
b. **Enrollment of study subjects**
c. **First MRI and clinical data received**

The current status of recruitment is indicated in Table 2. As has been noted in previous reports, recruitment was substantially delayed at the onset of the study due to complexities of the IRB approval process. All of the study categories have accrued participants, two have achieved the goal of 50 participants, and one other has come close to having achieved that goal. Recruitment of adults has continued to be problematic, in part because of relatively poor compliance of adults with attendance at NF clinics. At this point, it will be necessary to close the study to further accrual, in order to insure that the data that has been collected and will continue to be collected on enrolled participants can be analyzed. It may be necessary to combine some of the study cells in order to insure statistically meaningful data analysis, but having achieved 2/3 of our expected overall accrual we are confident that this will be successful.

Study Category		Number Recruited
Head & Neck	< 18 years old	50
	> 18 years old	17
Trunk & Extremity	< 18 years old	50
Externally Visible	> 18 years old	42
Trunk & Extremity	< 18 years old	28
Not Externally Visible	> 18 years old	22
Total		209

Table 2. Number of subjects recruited by study category.

d. **Review of clinical entry criteria**

Entry and exclusion criteria were reviewed in a meeting held in February 1999 at the Banbury Center in Cold Spring Harbor, N.Y. A follow-up meeting of the steering committee and participating clinical centers was held in Aspen, CO in June, 2000. No changes were made in the entry criteria at that meeting.

e. Test of inter-observer reproducibility of designation of tumor margins by MRI

Results of this study were reported last year. A paper on this topic is in press in American Journal of Radiology.

Task 3. ***Data Acquisition and analysis*** **– Months 13-42**

The current status of MRI receipt in shown in Table 3. Numbers of participants who have had 1, 2, 3, 4, or 5 scans received by the study are shown in Table 4.

Center	MRI scans
Children's Hospital	19
Children's Hospital Medical Center	15
Children's Hospital of Oklahoma	9
Children's Memorial Hospital	31
Children's National Medical Center	44
Guy's Hospital	19
Klinikum Nord Ochsenzoll	97
Mass General	3
National Cancer Institute	33
New Children's Hospital	16
Texas Children's Hospital	8
University of British Columbia	13
University of Utah	27
Washington University	35
Total	369

Table 3. Number of MRI scans received by site.

Number of scans per patient	Occurrence
1	115
2	80
3	16
4	9
5	2

Table 4. Occurrence of scans categorized by frequency. Any given patient only is counted once in this table.

Task 4. ***Interpretation of Data*** **– Months 43-48**

MRI Data

Analysis of sequential scans is now beginning, as we have accumulated sufficient such scans to permit analysis. The MRI data are analyzed by WorldCare, Inc. and the clinical examination data are stored in the NNFF international database. Prior to the analysis, we will merge the two databases by the examination dates. That is, for each MRI date, we will find the closest clinical examination date within 6 months of MRI date as the corresponding record. The primary objective of the study is to estimate the growth rate of plexiform neurofibromas for different subpopultions: adult versus children and tumors from different sites (head and neck, trunk and limbs - internal or externally visible). We will first examine the data graphically to determine the pattern of tumor growth (*e.g.,* linear or exponential growth). Mixed effects models will then be used to fit an overall regression models where the individual growth rate is considered as a random effect. An interaction term between age group and growth rate will be tested to determine whether growth rate differs between children and adults. Similarly, the interaction between tumor site and growth rate will be tested to determine whether the pattern of growth varies by site. Additionally, we will study the relationship between other clinical findings and tumor volume. The analysis will be done cross-sectionally with baseline (first visit) data and longitudinally with all available data. Variables with significant association with tumor volume will be included in multiple regression models to determine the independent effect of each variable.

Tumor Imaging Characteristics

Radiological analysis of plexiform neurofibromas has revealed that imaging characteristics differ between superficial and deep tumors. In order to characterize these differences more completely, sixty-six patients (median age 16.6 yr) with primarily superficial plexiform neurofibromas were compared to a similar group of 56 patients with deep plexiform neurofibromas (median age: 12.2 yr). All patients had axial STIR images, and coronal or sagittal STIR or T2-weighted images. The lesions were graded according to the following characteristics: location (chest, abdomen, pelvis, proximal or distal upper extremity, proximal or distal lower extremity, neck, scalp or face); presence or absence of symmetry, extension to skin surface, extension beyond subcutaneous tissues, border definition (good=1, moderate=2, poor=3); morphology (fascicular, nodular, or diffuse); maximal fascicular diameter; homogeneity (homogeneous=1, moderate=2, heterogeneous=3); and presence of increased vascularity.

Superficial neurofibromas were located in the trunk (18/66), extremities (22/66), and head and neck (26/66). Deep neurofibromas were located in the trunk (22/56), extremities (14/56), and head and neck (20/56). Superficial neurofibromas were more likely to be asymmetrical (odds ratio (OR): 6.9; 95% Confidence Interval (CI): 2,30) and extend to the skin surface (OR: 17; 95%CI:5.5,61). Border definition was more likely to be ill-defined in superficial neurofibromas (Figs. 1 and 2) with definition being poor in 43/66, moderate in

16/66, and good in 7/66. For the deep neurofibromas, border definition types were poor in 16/56, moderate in 25/56, and good in 15/56 ($P<0.0001$, Chi-square). Morphology of superficial neurofibromas was more likely diffuse (43/66, Figs. 1 and 2) than nodular (12/66, Fig. 3) or fascicular (11/66); deep neurofibromas were more likely nodular (25/56, Fig. 4) or fascicular (25/56) than diffuse (6/56) ($P<0.0001$, Chi-square). Signal characteristics of deep neurofibromas were more likely heterogenous (target like) (44/56) than homogeneous (4/56) or intermediate (8/56); superficial neurofibromas were infrequently heterogeneous (23/66) ($P<0.0001$, Chi-square). There was no difference in vascularity (both groups had increased vascularity in 34 cases, Fig. 2) or diameter of the fascicles or nodules between the two types of lesions (Wilcoxon Rank-Sum test).

We conclude that, unlike the typical target-like lesions along the course of major nerves seen in deep plexiform lesions, superficial plexiform neurofibromas are asymmetrical, have a more homogenous signal intensity, lack a nodular or fascicular morphology, have poorly defined margins, and are likely to involve the skin.

Figure 1. Axial and coronal STIR images of the midportion of the leg shows a superficial neurofibroma extending from the skin to the deep muscle fascia. The neurofibroma has diffuse morphology, homogeneous in signal intensity, poorly defined borders, and no evidence of increased vascularity.

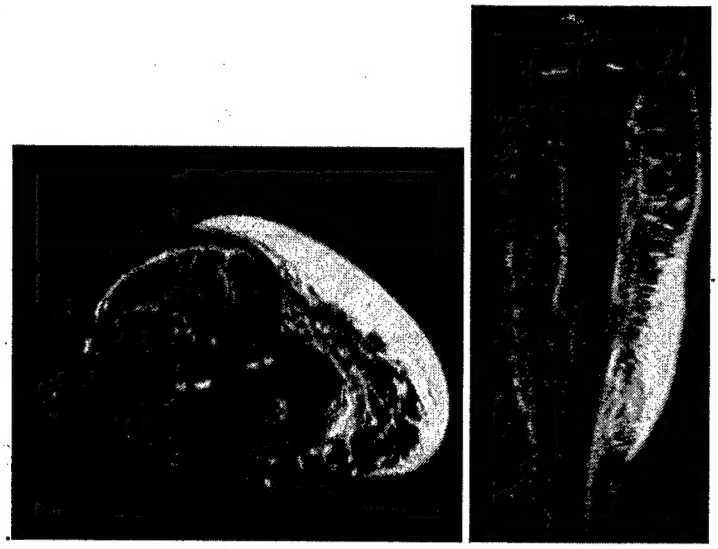

Figure 2. Axial STIR image through the leg of another patient shows another superficial neurofibroma extending from the skin to the muscles, with diffuse morphology, poorly defined margins, and homogeneous signal intensity. There is evidence of increased vascularity, with a dilated venous structure (arrow) within the lesion.

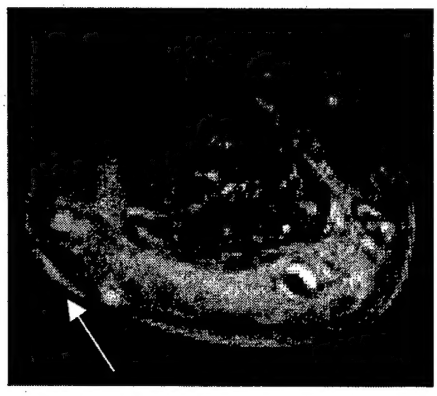

Figure 3. Axial STIR image through the gluteal region demonstrates a superficial neurofibroma with nodular morphology, inhomogenous signal intensity (target configuration, arrow), and well-defined margins.

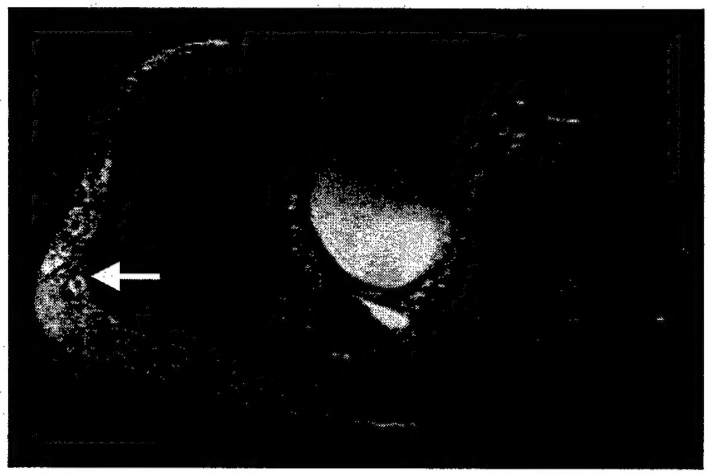

Figure 4. Deep plexiform neurofibromas of the sciatic nerve show the typical nodular, target-like configuration with well defined borders, which is typical of the deep lesions.

KEY RESEARCH ACCOMPLISHMENTS

- Publication of reproducibility study, demonstrating reliability of volumetric MRI approach (paper in press)
- Stable group of participating clinical centers in network
- Steady acquisition of MRI data and data analysis at WorldCare
- Initiation of statistical analysis of MRI data
- Identification of different imaging characteristics of superficial *vs.* deep plexiform neurofibromas

REPORTABLE OUTCOMES

1. Manuscripts: see references.

2. Presentations
   i) "Determination of Endpoints for Treatment of NF1" at NINDS-sponsored meeting "Neurobiology of Disease in Children: Neurofibromatosis 1" held in Victoria, BC, October, 2001. See reference list for published paper.
   ii) Summary of progress presented at National Neurofibromatosis Foundation Research Consortium meeting in Aspen, June, 2002 presented by Dr. Korf.
   iii) Presentation of summary of progress and proposal for formulation of NF Clinical Research Network at National Neurofibromatosis Foundation Clinical Symposium held at meeting of American Society of Human Genetics in Baltimore, October, 2002.

3. Patents, licenses: none

4. Degrees obtained: not applicable

5. Tissue Repositories: A repository of blood and tumor tissue is now established at Washington University, St. Louis. This repository was initiated as part of this project, but is now being used by treatment protocols for Pirfenidone and farnesyl transferase inhibitor, as well.

6. Informatics: The NF International Database has been modified to accommodate the specialized data collection required for use in this project. This database is open to investigators anywhere in the world (to input their own data, or query the database in a manner that preserves the confidentiality of patients.

7. Employment/research opportunities: not applicable

CONCLUSIONS

The study has brought together a set of clinical centers to recruit subjects with plexiform neurofibromas and has overcome significant challenges in obtaining IRB approval for participating centers. At this point in the study, it is planned to shift focus from continued accrual of new participants towards analysis of sequential data. If further accrual of participants is closed at this point, it will take an additional three years to complete the acquisition of MRI's from the most recently enrolled participants. From now forward, therefore, the focus of the study will remain on continued acquisition of MRI data from already enrolled participants, and continued radiological and statistical analysis of the MRI data. We are now seeking mechanisms whereby the network of clinical centers can continue to work together in support of additional clinical projects on NF1, including both natural history studies and clinical trials.

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